FABRICATION OF ELECTROSPUN DICALCIUM PHOPHATE ANHYDROUS AND CHITOSAN BASED SCAFFOLDS

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May 13, 2013

Certificate

This is to certify that the work in the thesis entitled as **fabrication of electrospun dicalcium phosphate anhydrous and chitosan based scaffolds** by S.Lipsita, with roll number 110CR0571, is a record of an original analysis work carried out by her under my supervision and guidance in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Ceramic Engineering.

SudipDasgupta

Acknowledgement

All of the attempts I have put forward within doing this particular project can have already been incomplete, if not for your type support of many individuals along with this particular initiate. Iwould really like to express my deep perception of appreciation to all of them. Principally, Iwould really like to say thanks to my supervisor on this venture, Prof. Sudip Dasgupta, Department of Ceramic Engineering, and National Institute of Technology Rourkela, intended for the incalculable contribution for the venture. This individual ignited me personally to be effective on the subject as well as provided useful information which usually assisted within performing the venture through different phases. We would furthermore want to accept the exemplary instruction, keeping track of as well as regular confidence during the entire span of this particular thesis.

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S.Lipsita

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Abstract

Dicalcium phosphate anhydrous (DCPA) powder was synthesized in the size range between 200-750 nm using calcium carbonate and phosphoric acid in ethanol solution. The X-ray diffraction pattern of the synthesized powder showed monetite as the major phase with some unreacted calcium carbonate as secondary phase. After 2hrs of stirring on reaction mixture in ethanol 3Wt% of each chitosan, poly ethylene oxide, dicalcium phosphate anhydrous was used for fabricating electrospun fibrous scaffold for bone tissue engineering. Glacial acetic acidcounts to be a suitable solvent for electrospining the suspension. The fabricated electrospun scaffold was characterized using X-ray diffraction and Fourier Transform infrared spectroscopy. Both X-ray diffraction and Fourier Transform infrared spectroscopy confirm the presence of chitosan, dicalcium phosphate and hydroxyapatite as major phases in the scaffold. Nanofibre morphology of the scaffold with average fiber diameter varying between 61 nm to 703 nm was observed from scanning electron micrograph of the electrospun scaffold.

CHAPTER#1

INTRODUCTION

1: INTRODUCTION:

Cells architectural or tissue engineering can be explained as a significant subject which aims at the development in the organic alternatives which can regain, maintain along with improve cells function. Attempts are actually created of this type to generate scaffolds which will assist cell growth, provide cell adhesion and mimic the extra cellular matrix in the cells. With the use of moving the strategy by prosthetic alternative to be able to regeneration, bone tissue engineering employing osteo-inductive along with osteo-conductive scaffolds might provide a feasible method to defeat the reconstruction in the huge bone defects. These types of scaffolds may be geared up by biodegradable polymers, ceramic or perhaps composites that may contain the two ceramic levels along with polymer bonded. On the list of various kinds scaffolds electrospun works have an edge over some others. Coelectrospinning, pairing polymers along with bioactive substances since hydroxyl apatite, can certainly further increase the biometric attributes of nanofibrous scaffolds along with increase incell connection and bone extracellular cell matrix (ECM).

Bone tissue can be a unique cell that is triphasic which possesses cellular parts, hydrated extracellular organic and natural matrix, along with an extracellular nutrient phase, that's largely composed of calcium supplement phosphate typically in the form of hydroxyapatite.

Significance about ECM:

- ECM functions being a scaffold to deliver assistance along with hold the cells with each other
- Handle the tissue’s framework
- Regulate cellular function.
- It interacts with all the next cellsboth mechanically, biologically.

Collagen makes energy for the cells and also facilitates cellular anchoring. The particular ultrathin fibers created by electrospining include high surface, linked porosity to follow along with the framework of indigenous ECM. It also boosts the chances of cell and manmade extra

cellular matrix relationship. Bone tissue graft getting bonelike appearance may be created from electrospun nanofibrous scaffolds.

A substantial quantity of producing techniques is actually looked into to be able to fabricate tiny or perhaps fibrous matrices which are in nano scale range, which include fiber drawing, synthesis directed by template, separation of phase, along with electrospining. Amongst these types of tactics, electrospining has been acknowledged because simplest and lowest priced ways to fabricate ultrafine fibers. Many experts have utilized to be able to fabricate nanofibers by various man made or perhaps natural polymers. Organic polymers are generally typically preferred over man made polymers because with their confirmed bio-compatibility along with bioresorbability.

Collagen along with chitosan is generally a couple naturally taken biopolymers that are common inside cells architectural. It has been helpful for fabrication of nanofibers along with diameters in the selection of 100 nm. Chitosan has been shown to be biodegradable, biocompatible along with non-antigenic. Chitosan was obtained from the deacetylation of chitin; a polysaccharide resulting from the exoskeleton of crustaceans has surfaced being a promising candidate pertaining to bone cells architectural, largely because of its biocompatibility along with structural likeness to bone ECM. Chitosan scaffold has been extensively looked into with the bone cells architectural and observed to improve bone sourcing the two inside vitro along with inside vivo.

A chance of fabrication of chitosan-based nanofibers along with controlled dimensions and alignment in a particular direction to create nonwoven exercise mats or perhaps 3-D porous set ups will give an almost infinite supply with the development of natural polymer-based ECMs. Presently, some attempts are actually created to put together chitosan-based nanofibrous set ups through electrospining. Chitosan nanofibers that has a imply size of 40 nm had been experimentally noted. Polyethylene oxide (PEO) was incorporated inside chitosan to be able to fabricate ultrathin a mix of both nanofibers. This demonstrated that the spinability of chitosan suspension depends powerfully within the bulk proportion of chitosan to be able to poly ethylene oxide, along with nanofibrous set ups along with dietary fiber diameters ranging from 50 to be able to 180nm had been achieved in the alternative that has a poly ethylene oxide-to-chitosan bulk proportion of just 1: 1. Poly ethylene oxide is utilized to lessen the viscosity of chitosan alternative so that the alternative is actually spinable in high polymer bonded levels.

DCPA (dicalcium phosphate anhydrous) is one of the a little acidic along with soluble calcium supplement phosphate levels. Presently it has taken a significant place in the powdered ingredients components of self-hardening calcium supplement phosphate pastes, that's for skeletal fix. It has a triclinic device mobile or portable with all the lattice details; a= 6. 910, b= 6. 627, c= 6. 998 A^0 along with α= 96. 340, β= 103. 820, γ= 88. 330. It has a density of 78 g/cm3. $CaHPO_4$ may be synthesized through dehydration process. $CaHPO_4$. $2H_2O$ (dicalcium phosphate dihydrate, DCPD) often known as brushite powders may be become single-phase $CaHPO^4$ through dehydration. Two strategies to dehydration may be implemented.

- First approach: $CaHPO^4$synthesis employs brushite powders as starting up materials. The process contains these steps- dehydrating 300 gm of brushite ($CaHPO_4$. $2H_2O$) inside 4ml of 0. 07 M H3PO4 alternatives along with heating this pertaining to 74 hrs.Then the synthesized $CaHPO_4$ powders were filtered.
- Second approach: Dehydrating brushite powders to obtain $CaHPO_4$ simply by heating system brushite in the static fresh air atmosphere more than a temperatures selection of 200–250^{0}C.

Both of these dehydration techniques develop the pursuing negatives:

(a) In conditions of ceramic powdered ingredients scientific discipline: it will be very difficult to be able to significantly modify the particle size and shape supply that is certainly within the initial brushite powders. Most of the manmade brushite powders exhibit huge (i.e10–130 mm) tabular, needle-like or perhaps rectangular prismatic deposits. When the sodium attention is actually huge acicular deposits may also be created. That huge particle dimensions, that's nearly 15–40 μm is the principal barrier of currently available industrial $CaHPO_4$ powders. As a result of this kind of tabular or perhaps acicular amazingly appearance inside brushite, monetite synthesis procedures which derive from the dehydration of brushite are not capable of creatingsub micrometer particles.

Quite a few attempts are actually created to acquire a few brand-new powdered ingredients synthesis techniques, which would provide a reduction in particle dimensions of dicalcium phosphate. Some of them are:

- A membrane layer tiny dispersion mixing up process which employs $CaHPO_4$ particles inside oil-in-water program along with water-in-oil program.
- A alternative of H_2O along with cyclohexane comprising surfactants similar to polyoxyethylene-8-dodecyl ether (C12E8) along with n-pentanol since co-surfactant can certainly create nano dimensions particles.

Most efficient opportunity for synthesizing monetite is within ethanol along with phosphoric acid inside area temperatures. Monetite synthesis inside ethanol comprising a few amounts of phosphoric in area temperatures, takes place using the following equations:

$$CaCO_3(s) + 2H_3PO_4(aq) \longrightarrow Ca(H_2PO_4)_2.H_2O\ (s) + CO_2\ (g)$$

$$Ca(H_2PO_4)_2\ .H_2O(s) \longrightarrow CaHPO_4(s) + HPO_4^{2-} + 3H^+\ (aq) + OH^-$$

By this method pure monetite nano materials can be produced. These nano sized particles provide relatively better performance than the conventional materials because of its high surface to volume ratio. These have controlled morphologies which make it suitable for many biomedical applications. Monetite is used in powder form in some of the toothpastes and chewing gums. It is also used to regulate acidityin food processing industry, dough modifier, anti-caking agent and emulsifier. It can be used as reasonable bone replacement material due to its osteo-counductivity and osteo-inductivity.

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

Bone tissue defects is usually reconstructed by making use of allografts, autografts and also alloplastic material nevertheless the autografts are usually constrained within provide and also related generally using donor website morbidity. Prosthetic supplies and allografts commonly show very poor integration with all the encompassing host bone, displacement and also fragmentation [1-3]. The interface in between the host cells and grafts offers mechanical properties which often stay impaired in comparison with autografts. It is described the earlier mentioned circumstance occurs as a result of deficiency of fresh bone creation across the junction [4]. As a result, some sort of bioactive scaffold will be engineered which brings together both natural and organic and also inorganic interphase involving bone tissue along with the regenerative potential from the periosteum. It can be some sort of probable means to fix the lack of osteointegration involving autograft replacements [4].

Structure assemble which has already been efficiently engineered will probably physically, chemically and also structurally copy the local further extra cellular matrix cells and its important mechanical properties. Though morphologically scaffolds which are electrospun resemble the fibrous composition from the e, the mechanical properties cause them fewer ideal to be used asbone analogs. Crosslinking may perhaps increase the mechanical properties and also tunethe assemble toincreasethe approximate fibrous tensile properties involving bone ECM [5, 6, 7].

The particular electrospining practice must be optimized regarding bringing in genuine chitosan (CTS) and also chitosan-hydroxyapatite dietary fiber simply because recent processes work with poly ethylene oxide (PEO), fiber-forming excessive molecular fat additives. This may slow down multi-layer increase involving tissues [8]. Scientific studies have shown the operation ailments and also method setting range significantly from material method to a different. This will depend around the material and the collection of solvent. Physical and also element guidelines like electric conductivity, viscosity and also polymer bonded concentration impact the morphology and also formability involving electrospun material. With electrospining chitosan the main complication may be the poor solubility and the excessive viscosity involving chitosan aqueous

remedy. Lower polymer bonded concentration usually do not consist of ample material that may generate stable strong material. As the polymer bonded concentration will increase the primary interchain links involving chitosan substances inside the solution will increase easily and also gets to an essential importance being created some sort of 3-D community composition, a highly viscous gel, generating the perfect solution unspinable. The particular viscosity of the suspension is usually an important issue of which impacts suspension morphology and also spinability involving as-spun material [9, 10, 11, 12]. A work has shown membrane layer involving nanofibers which often has 40 wt% PEO completely lost its primary fibrous composition after immersion within water regarding 1 week. In contrast to the membrane layer made from nanofibers using nearly 10 wt% PEO stores it is morphology within drinking water over the very same period of time[13-14]. Alignment involving electrospun material offers useful significances within cell engineering, specifically regarding renovating and also improvement involving the two engineered cardiovascular system flesh and also local flesh [15-17]. With regards to the rotating swiftness some sort of rotating collector may be used to generate fibrous buildings having a certain amount of conjunction, while described inside the literature [14]. The particular fibrous composition had been enhanced by the launch from the cosolvent, and the material accumulated in the two standing and also rotating collectors have been almost bead-free.

In drugs delivery applications electrospun nanofibers are most popularly used. The process of loading of drug can be easily applied. High applied voltage is used in electrospining process but it has little effect on the activity of drug. In comparison to the bulk material the nanofibers give drug have higher total release rate for its short diffusion passage length along withhigh specific surface area. The modulation of nanofiber morphology, porosity and compositioncontrol the profile finely [18, 19, and 20].For developing conventional and new pharmaceutical products nanofibers are the best choice. Bio compatibility, nontoxicity, bio degradability and anti-bacterialactivity are some of the most vital biological properties which make nanofibers most favorable option. For improving absorption of drugs, cell proliferation, enzyme inhibition and wound healing, nanofibers are indispensable.[21, 22, 23].

CHAPTER # 3

EXPERIMENTAL METHODS

3. MATERIALS AND METHODS

3.1: Materials:

Calcium carbonate powder[Calcite ($CaCO_3$)] powders used in this experiment were selected on the basis of their mean particle size (i.e.submicrometer) and morphology. The precipitated calcite ($CaCO_3$) powder was used in this study. Ortho phosphoric acid (density of 1.75 ml/gm) was used as P source, ethanol and hydrochloric acid as solvent.

Chitosan [percentage deacetylation (90%), molecular weight 6000 Da] and polyethylene oxide (PEO) were purchased from Sigma Aldrich (USA).

3.2: Synthesis of DCPA:

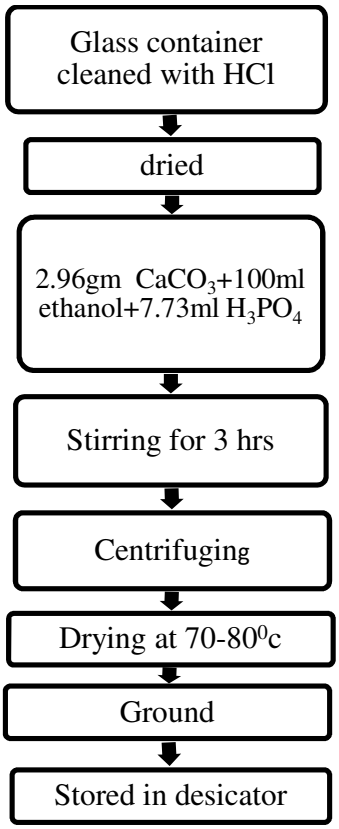

Fig 1: Flow Chart for synthesis of DCPA

To yield only single-phase $CaHPO_4$, 100 mL of ethanol was first placed into a 100 ml capacity beaker with a magnetic stir bar which was Teflon coated. 2.96 g of $CaCO_3$ powder (0.0296 mol Ca) was then added into ethanol and the resulting opaque suspension was magnetically stirred at room temperature for 5 min. Finally, 4 mL of H_3PO_4 (0.0592 mol P) was added into the calcite suspension. The glass beaker was tightly capped and the contents were stirred at room temperature for 3 hr. At the end of 3 hr, the beaker was opened and the precipitates were filtered. Then it was washed with 25 ml of ethanol, and kept for centrifuging. The centrifuge bottles having the precipitates were finally dried overnight at 70 -80^oC in a static air oven. Thestarting Ca/P molar ratios weretested over the range of 0.5–1.0, whereas the stirring times at room temperature were kept from 30 min to 4 h.The dried sample was ground and stored in a desiccator.

3.3: Preparation of solution for electrospining:

- Two percent chitosan solution and 2% PEO solution were first prepared separately by dissolving chitosan or PEO in glacial acetic acid.
- The chitosan and PEO solution was stirred overnight.
- The chitosan and PEO solutions of different proportions were then mixed to obtain the mixtures with weight ratios of 60/40 of chitosan and PEO, and the resultant mixtures were stirred for 5 hr.
- Solutions are then centrifuged.
- To this solution 2wt% of DCPA was added.

3.4. Electrospining of nanofibers:

The suspension well prepared intended for electrospining had been raised on right into a 5ml syringe which has a pipette tip of 0. 5mm in size. The suspension had been driven through the law of gravity and the rate had been controlled through the tilt position of the syringe. Temporarily, a DC voltage of 20–25 KV had been employed relating to the syringe along with a collector (cylindrical in nature) insured with the lightweight aluminum foil. The particular cylindrical tube experienced a size of 7 cm also had been driven by a DC electric motor using controllable rate. The gap relating to the syringe tip and the collector had been 17–20 cm. During the process of electrospining, the droplet at the syringe tip had been separated by way of a repugnant push set through the charge from the droplet, as well as shaped a jet of cone-like

appearance journeying towards the collector. During this time the solvent evaporated as well as polymer bonded fibers placed on the collector in the form of a nonwoven fibrous sparring floor. All of the electrospining experiments had been carried out at room temperature. The particular unique nanofibers had been dried up underneath vacuum at room temperature. The particular morphology of the nanofibers had been looked at by scanning Electron Microscope from the augmenting voltage of 10 KV. The normal size of electrospun nanofibers had been driven by computing the diameters of the nanofibers from different places in a Scanning Electron Microscope image.

CHAPTER 4

CHARACTERIZATION

4. CHARACTERIZATION:

4.1: x-Ray Diffraction (XRD) of DCPA:

Synthesized powder of DCPA was characterized by powder X-ray diffraction. Powder samples for the XRD analysis were first finely ground in a mortar pestle by using an agate pestle. A single-crysral quartz sample holder was used for holding the samples.It was first cleaned with ethanol and then the samples were set into the quartz sample holders to form a thin layer. This filling of powder sample was followed by tapping to remove the excess of powder. The X-ray diffractometer was operated at 30 mA and 40 kV by using CuKα radiation, which was mostly monochromated. XRD was done at 10-80^0 scanning range.XRD data were collected with a step size of 0.5 and each step had apreset time of 1 minute.

4.2: Fourier Transform Infrared Spectroscopy (FTIR) of DCPA and scaffold:

The samples for FTIR were prepared by grinding the samples into fine powder in a mortar. Then it was mixed with moisture-free and pure KBr powders in a ratio of 1:100, followed by forming apellet. A thin pellet of nearly 1cm diameter was prepared by using a uniaxial cold press. Hundred and twenty-eight scans were performed at a resolutionof 3 cm-1. The spectra were collected on a Varian Inc. FTS3000 Excalibur FTIR spectrometer which is equipped with a detector of Deuterated Triglycine Sulfate (DTGS) and a beam splitter of KBr. The spectra were recorded at resolution of 4 cm^{-1}in the range of 400-4000 cm^{-1}in transmission model.

4.3: Particle size determination of DCPA:

Particle size determination was done by Dynamic Light Scattering. Dynamic light scattering can be used to measure the particle size distribution profile of small particles in suspension by measuring the variation in scattered light. Here the particle undergoes Brownian motionso the distances between the scatterers change with time in the solution.

In 10mlof water 0.02gm of DCPA powder was taken. It was ten kept for sonication to get a dispersed phase in an ultrasonicator. After three minutes of ultrasonication the desired dispersed phase was obtained. A little amount of this solution was then taken for the sample holder and the particle size determination was done.

4.4: X-Ray Diffraction of scaffold:

XRD was performed using a Siemen powder diffractometer using conventional Bragg geometry, with CuKα source (0.154 nm). Scans were acquired from 10-80^0 scanning range with a step size of 0.5 and 1 s dwell time per point.

4.5:Scanning electron microscopy (SEM) of scaffold:

Scanning Electron Microscopy was used for ultrastructural analysis. For the analysis experimentally prepared scaffold samples were sputter coated with carbon. The samples were viewed and digitally photographed in a Nova nanosem 450field emission scanning electron microscope (FESEM) equipped with a Bruker Energy Dispersive Spectrometer at 5 kV with the SE2 detector using a 30 micrometer final aperture.

4.7: Energy dispersive X-ray spectroscopy (EDS):

An FESEM which is equipped with an Energy Dispersive X-ray Spectroscopy (EDAX) system was used to assess elemental composition of the scaffolds. X-ray spectra using a 60 μm final aperture were taken at 10 kV. EDS was performed by using the FESEM at an acceleration voltage of 10 kV.

CHAPTER 5

RESULTS AND DISCUSSIONS

5: RESULTS AND DISCUSSION:

5.1: XRD analysis of DCPA:

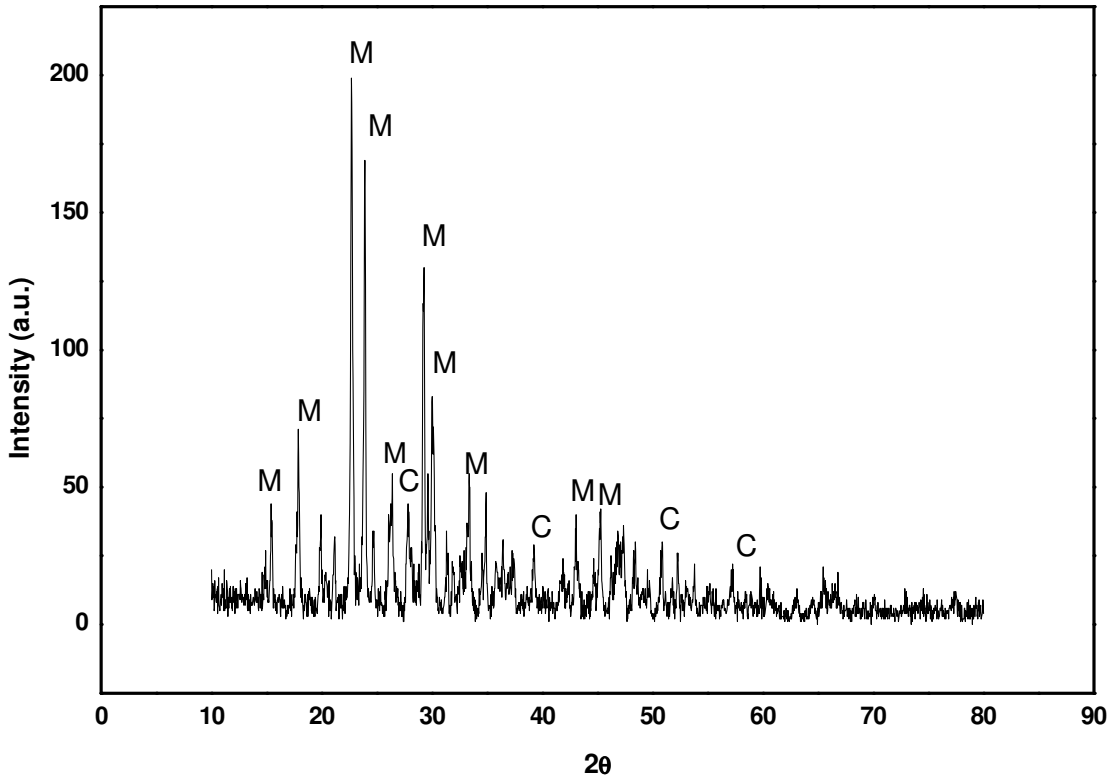

Fig 2.X-ray diffraction pattern of synthesized dicalcium phosphate anhydrous powder (M→monetite, C→ calcite).

X-ray diffraction pattern of synthesized dicalcium phosphate anhydrous powder is shown in figure (2). Monetite, $CaHPO_4$appeared as a major phase in the synthesized powder. Small fraction of unreacted calcium carbonate was also found in phase composition of the synthesized powder. These samples were synthesized afterstirring for only 3hr in ethanol at room temperature. 4hr stirring did not produce any noticeable changes in the phase composition of the synthesized powder, thus reaction was completed within 3 hr of stirring. So, stirring thereaction mixture beyond 3hr was notnecessary. The pH of the solution at the end of 3 h of stirring at room temperaturewas nearly constant of about 4. The highest intensity peak was obtained at around 30^0 which consist of monetitephase.

5.2: Composition of the suspension for the electrospinning:

SOLVENT	DCPA Wt%	PEOW t%	CHITOSAN Wt%	ELECTROSPINABILITY	NANO FIBROUS MESH
Triflouro acetic acid	3	3	3	Yes	No
Trifluoro acetic acid	2	3	3	yes	No
Trichloroacetic acid (99%)	3	3	3	Yes	No
Trichloroacetic acid(80%)	2	2	2	No	No
Chloroacetic acid(85%)	2	2	3	No	No
Cloroacetic acid(90%)	3	3	3	No	No
Acetic acid	3	3	3	yes	No
Acetic acid	0	3	3	yes	No
Glacial aceticacid	3	3	3	yes	Yes
Glacial acetic acid	2	2	3	yes	Yes

Table 1: composition of solution for electrospinning

5.3: FTIR analysis of DCPA:

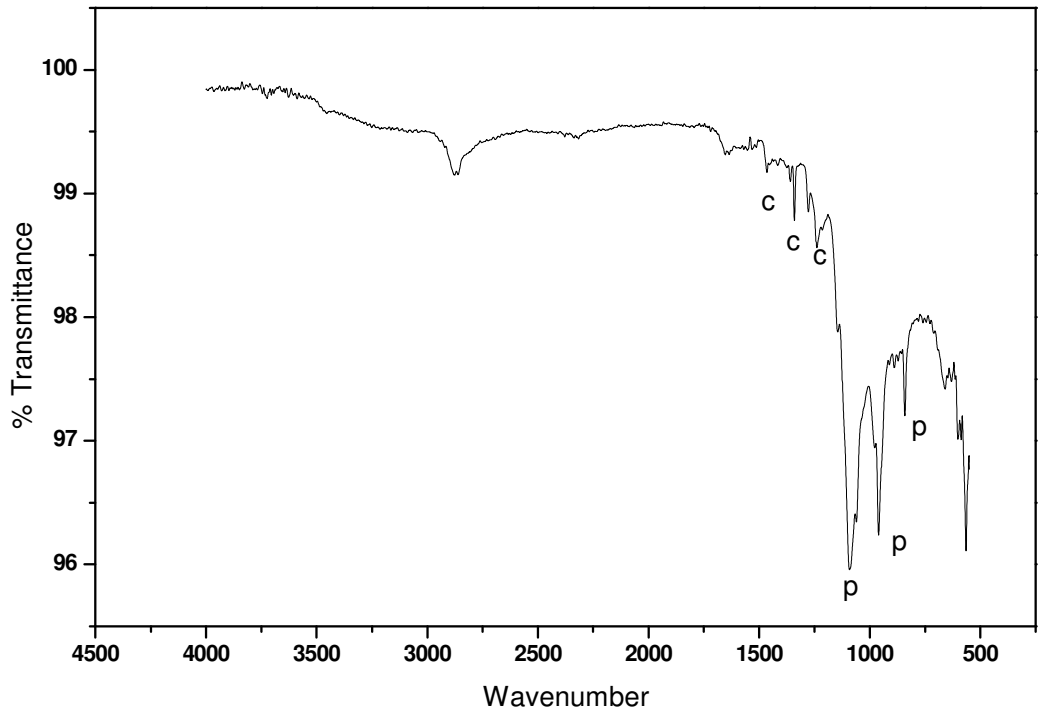

Fig.3. FTIR of DCPA:

The FTIR spectra presented above in figure 3, shows all the characteristic infrared band of $CaHPO_4$. The phosphate groups in DCPA showed characteristic FTIR bands between 900-1100 cm^{-1} and 500-600 cm^{-1}. Traces of carbonate peaks also appeared around 1360 cm^{-1} due to the presence of unreacted calcium carbonate. The presence of labile hydroxyl bond around 2876 cm^{1}revealed the presence of water molecule in fraction of transformed $CaHPO_4$. H_2O.

5.4: Particle size distribution:

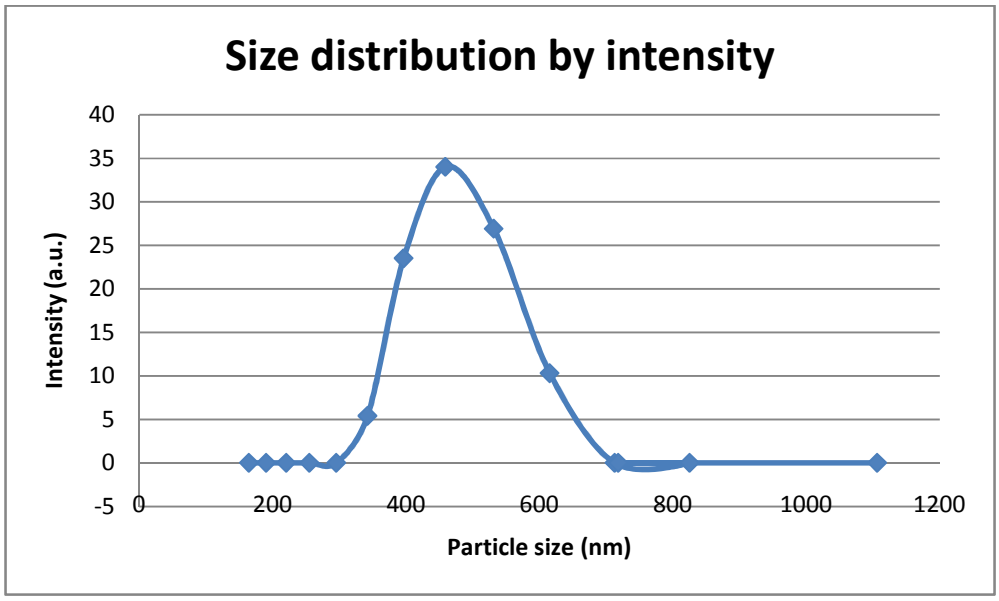

Fig 4: Particle size distribution by intensity

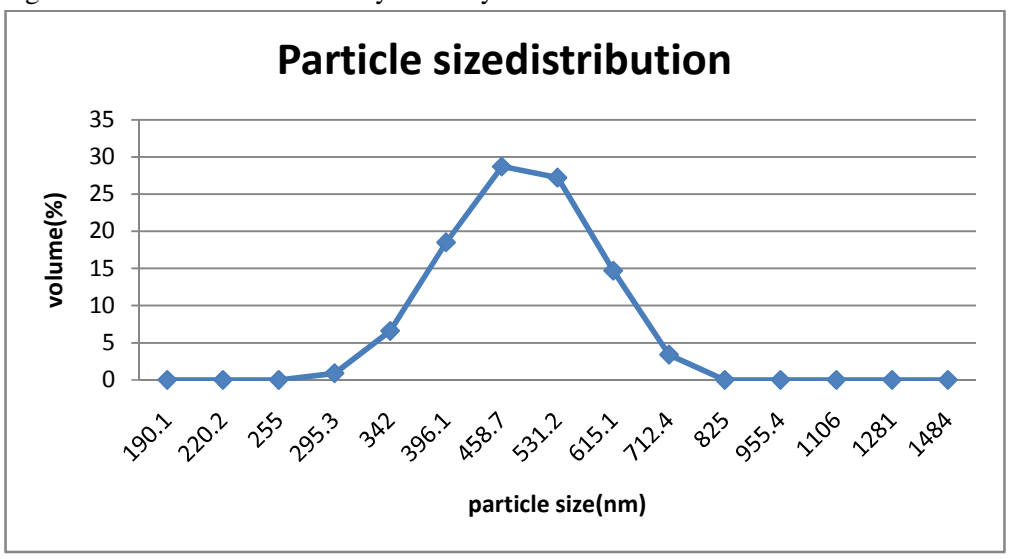

Fig 5: particle size distribution by volume percent

The particle size distribution of synthesized dicalcium phosphate anhydrous obtained through both intensity and volume percent distribution is shown in figure 2. Average particle size of DCPA was found to be 460 nm and the distribution wasunimodal. These shows that synthesized dicalcium phosphate anhydrous nano powder were nearly mono dispersed or having a narrow particle size distribution.

5.5: XRD of scaffold:

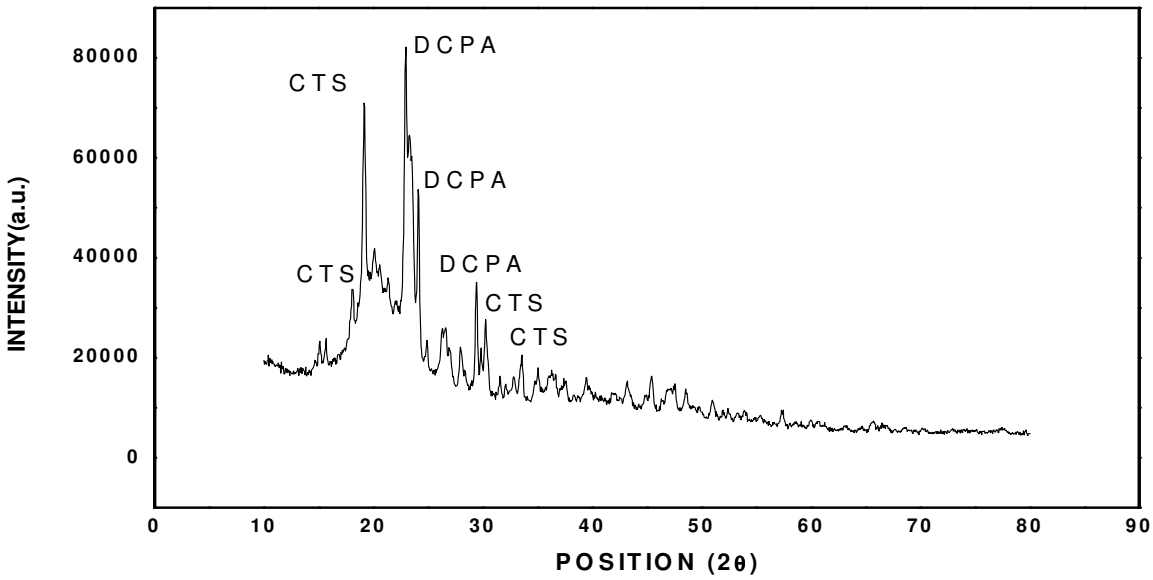

Fig 6: X-Ray Diffraction pattern of scaffold:

The powder X-Ray Diffraction pattern of electrospun scaffold is shown in figure (6). Both dicalcium phosphate anhydrous chitosan peaks were detected in the scaffold and some fraction of hydroxyapatite also appeared in the X-Ray Diffraction pattern. As dicalcium phosphate anhydrous is highly susceptible tohydrolysis to hydroxyapatite in the suspension mixture in presence of acid and water, some amount of dicalcium phosphate anhydrous was readily hydrolyzed to hydroxyapatite.

5.6: FTIR of scaffolds:

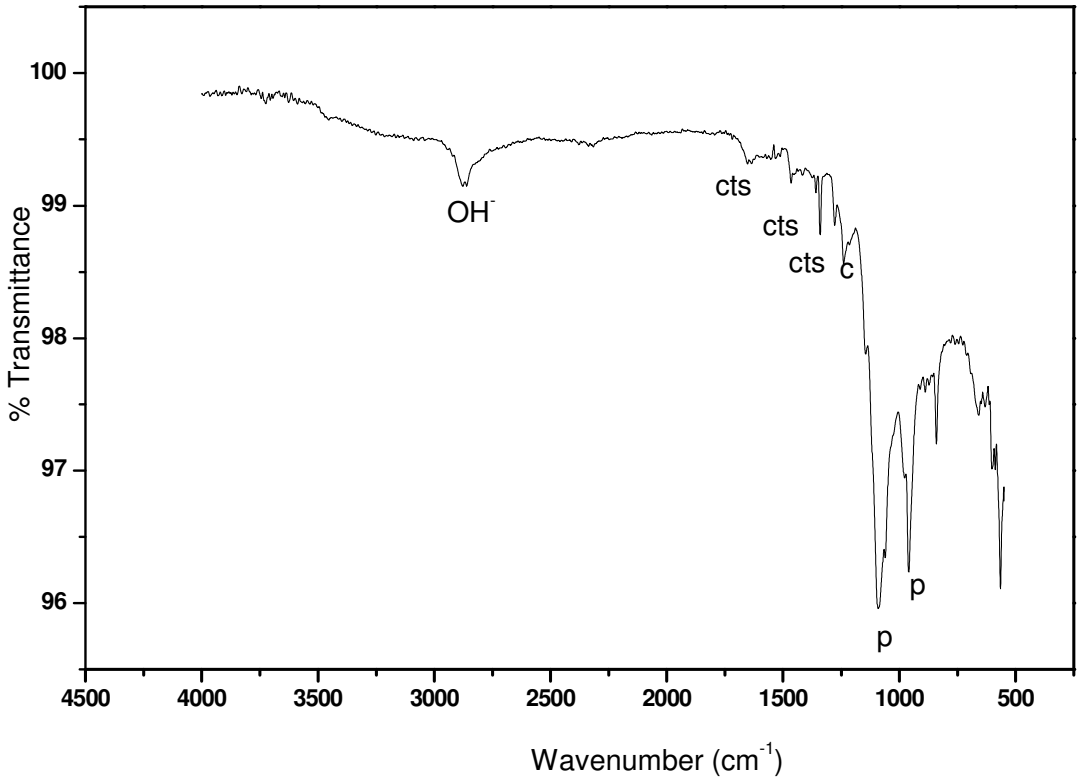

Fig 7: FTIR of scaffolds:

Fourier Transform Infrared Spectroscopy spectra of the fabricated electrospun scaffold are shown in figure (7). Stretching and bending vibration of phosphate from calcium phosphate phases appeared between 800-1100 $cm^{-1.}$ At 1340 $cm^{-1}$the peaks of carbonate appeared due to the presence of hydroxyl apatite phase. The stretching vibration of C-O bond in chitosan was detected at around 1465 cm^{-1}whereas the presence of amine bonds ($-NH_2$) in chitosan was observed at around 1653 $cm^{-1.}$ The presence of labile hydroxyl group at around 2876 $cm^{-1}$was primarily due to the converted hydroxyapatite groups.

5.7: SEM of scaffolds:

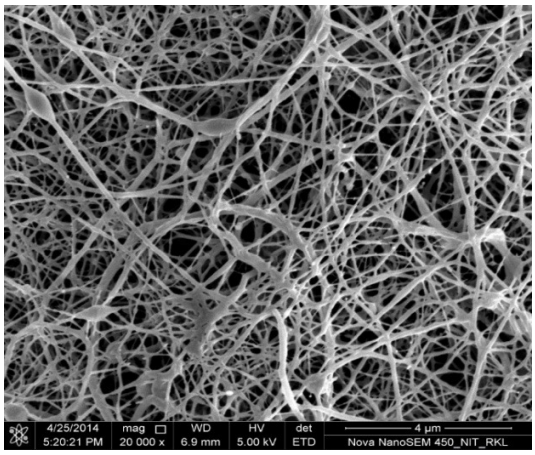

Fig 8(a) SEM images of Nanofibrous scaffolds

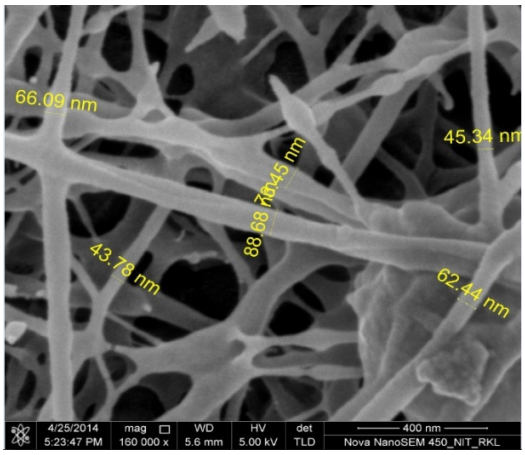

Fig 8(b) SEM images showing the diameter of nanofibers

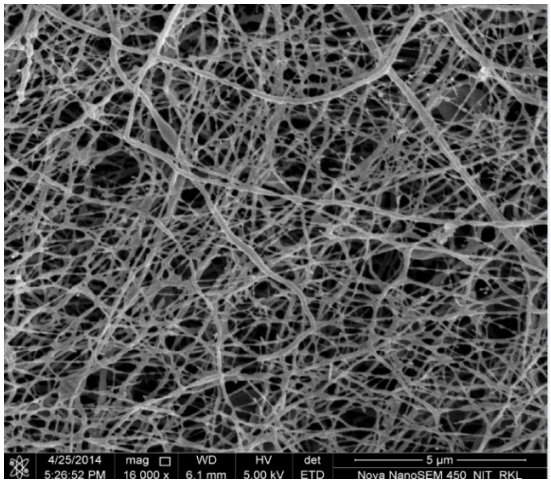

Fig 8(c)

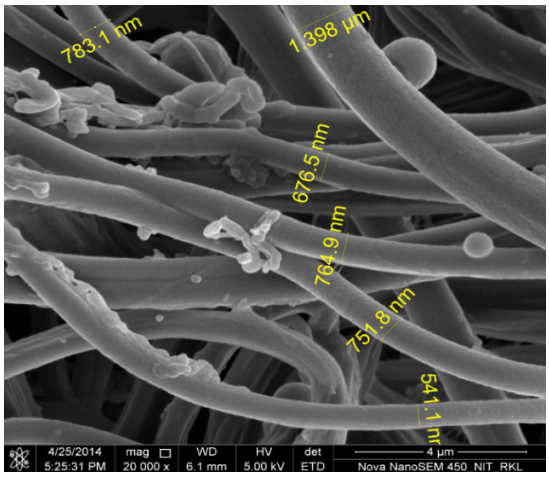

Fig 8(d)

The SEM images shown above are the images of the as-spun products. It shows the morphology of unwoven fibrous mesh. The average diameters of the nanofiber estimated from the images varied between 61.266nm and 703.48nm. This variation in diameter was seen because images were taken at different points.This non uniformity in diameter of electrospun fiber can be eliminated by using some polar and non polar cosolvents.

The extra cellular matrix of bone is comprised of hierchical assembly of nanofibrous collagen with hydroxyl apatite nano crystals dispersed in it. In this research we have fabricated

nanofibrous chitosan mesh embedded with osteo-inductive dicalcium phosphate ($CaHPO_4$) crystals. As nano fiber would stimulate bone tissue regeneration and the presence of osteoinductive$CaHPO_4$would help in differentiation of bone cell. This kind of electrospun scaffold can work as an ideal bone substitute material.

5.8: Energy dispersive X-ray spectroscopy (EDS) of scaffolds:

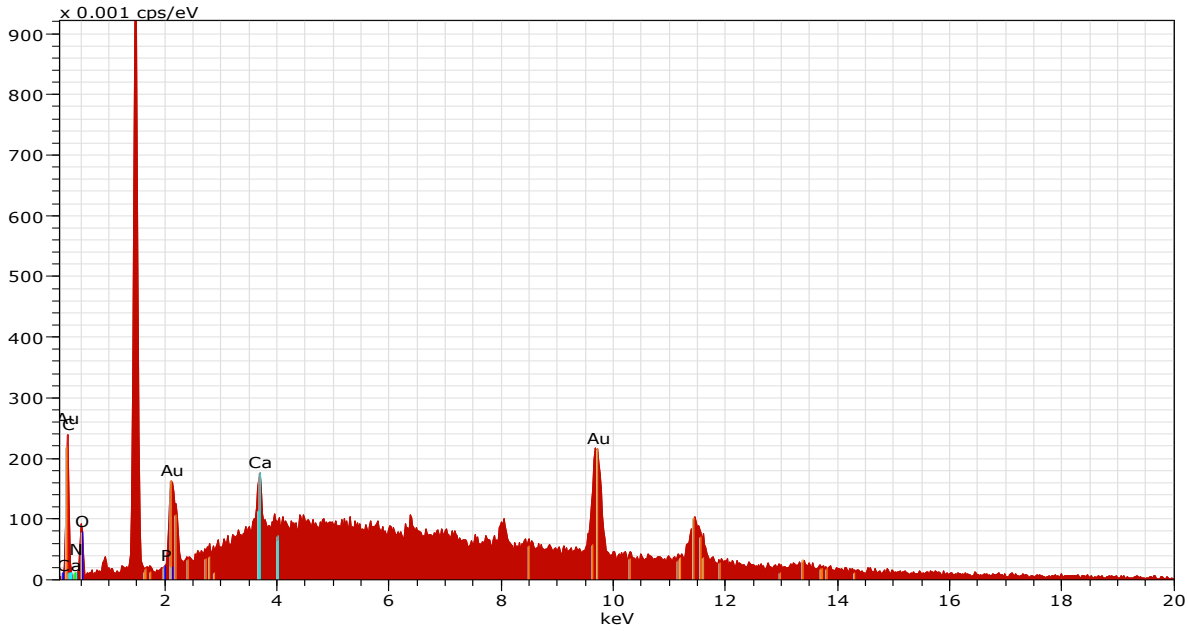

Fig 9: EDS of scaffolds

ELEMENT	SERIES	Wt%
Carbon	K series	60.75
Oxygen	K series	34.67
Nitrogen	K series	1.33
calcium	K series	1.94
phosphorous	K series	1.3

TABLE 2 : Elemental composition of electrospun scaffold

EDS was used to determine the elemental composition of the electrospun scaffold. Chitosan – DCPA scaffolds showed large peaks for carbon and oxygen and a small peak for nitrogen, indicating three of the main components of chitosan. As the fibers contain 3 wt% of DCPA, additionally the elemental composition showed some amounts of calcium and phosphorus. The fiber contains 60.75 wt% of carbon, 34.67 wt% of oxygen, 1.33 wt% of nitrogen, 1.94 wt% of Ca and 1.3 wt% of phosphorous. The large peak of alumina was obtained because the fibers were deposited on Al substrate.

CHAPTER # 6

CONCLUSIONS

6. CONCLUSIONS:

Chitosan and dicalcium phosphate anhydrous basednano fibers with an average diameter of 460 nm were successfully fabricated by electrospining at a higher voltage. The nano fibers were deposited as a nonwoven membrane. It was not possible to fabricate nano fibers from all suspension containing chitosan, polyethylene oxide and dicalcium phosphate by electrospining. It depended on the kind of solvent used and the viscosity of the suspension, which further depended on the amount of polyethylene oxide added. The fabricated electrospun scaffolds was characterized using X-ray diffraction and Fourier Transform infrared spectroscopy. Both confirmed the presence of chitosan, dicalcium phosphate and hydroxyl apatite in the scaffold. Scanning electron microscopy confirmed the formation of nanofibrous structure. These electrospun Chitosan and Dicalcium phosphate anhydrous based scaffolds can mimic the native extra cellular matrix of bone which helps in the reconstruction of bone defects in the field of bone tissue engineering.

CHAPTER# 7

REFERENCES

7. REFERENCES:

[1] Ransford AO, Morley T, Edgar MA, Webb P, Passuti N, Chopin D, et al. Synthetic porous ceramic compared with autograft in scoliosis surgery. A prospective, randomized study of 341 patients. J Bone JtSurg Br 1998;80:13e8.

[2] Banwart JC, Asher MA, Hassanein RS. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine (Phila Pa 1976) 1995;20:1055e60.

[3] Borden M, Attawia M, Khan Y, Laurencin CT. Tissue engineered microsphere based matrices for bone repair: design and evaluation. Biomaterials 2002;23: 551e9.

[4]Electrospun hydroxyapatite-containing chitosan nanofiberscrosslinked with genipin for bone tissue engineering Michael E. Frohbergha, Anna Katsmanb, Gregory P. Bottaa,c, Phillip Lazarovicia,d, Caroline L. Schauere, Ulrike G.K. Wegste,1, Peter I. Lelkesa

[5] Li WJ, Laurencin CT, Caterson EJ, Tuan RS, Ko FK. Electrospunnanofibrous structure: a novel scaffold for tissue engineering. J Biomed Mater Res 2002; 60:613e21.

[6] Zhang K, Qian Y, Wang H, Fan L, Huang C, Yin A, et al. Genipin-crosslinked silk fibroin/hydroxybutyl chitosan nanofibrous scaffolds for tissue-engineering application. J Biomed Mater Res A 2010;95:870e81.

[7] Chen H, Huang J, Yu J, Liu S, Gu P. Electrospun chitosan-graft-poly (epsilon caprolactone)/poly (epsilon-caprolactone) cationic nanofibrous mats as potential scaffolds for skin tissue engineering. Int J BiolMacromol 2011;48: 13e9.

[8] Electrospun chitosan-based nanofibers and their cellular compatibility Narayan Bhattaraia, Dennis Edmondsona, OmidVeiseha, Frederick A. Matsenb, MiqinZhanga,b,aDepartment of Materials Science & Engineering, University of Washington, Seattle, WA 98195-2120, USA bDepartment of Orthopaedics and Sports Medicine, University of Washington, Seattle, WA 98195-6500, USA

[9] Zhang Y, Zhang MQ. Synthesis and characterization of macroporous chitosan/calcium phosphate composite scaffolds for tissue engineering. J Biomed Mater Res 2001;55:304–12.

[10] Jayaraman K, Kotaki M, Zhang YZ, Mo XM, Ramakrishna S. Recent advances in polymer nanofibers. J NanosciNanotechnol 2004;4:52–65.

[11] Reneker DH, Chun I. Nanometre diameter fibres of polymer, produced byelectrospinnin g. Nanotechnology1996;7:216–2 3.

[12] Yarin AL, Koombhongse S, Reneker DH. Bending instabilityinelectrospinning of nanofibers. J ApplPhys 2001;89:3018–26.

[13] Kumar MNVR. A review of chitin and chitosan applications. React FunctPolym 2000;46:1–27. React FunctPolym 2000;46:1–27.

[14] Ohkawa K, Cha DI, Kim H, Nishida A, Yamamoto H. Electrospinning of chitosan. Macromol Rapid Commun 2004;25: 1600–5.

[15] Li D, Wang YL, Xia YN. Electrospinning of polymeric and ceramic nanofibers as uniaxiallyaligned arrays.NanoLett 2003;3:1167–71.

[16] Zussman E, Theron A, Yarin AL. Formation of nanofiber crossbars in electrospinning. ApplPhysLett 2003;82: 973–5.

[17] Khil MS, Cha DI, Kim HY, Kim IS, Bhattarai N. Electrospunnanofibrous polyurethane membrane as wound dressing. J Biomed Mater Res B 2003;67B:675–9.

[18] Duan B, Dong CH, Yuan XY, Yao KD. Electrospinning of chitosan solutions in acetic acid with poly(ethylene oxide). J BiomatSci-Polym E 2004;15:797–811

[19] Bhattarai SR, Bhattarai N, Yi HK, Hwang PH, Cha DI, Kim HY. Novel biodegradable electrospun membrane: scaffold for tissue engineering. Biomaterials 2004;25:2595–602.\

[20] Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Compos SciTechnol 2003;63:222353.

[21] Malette WG, QuigleyH, Adickes ED. Chitin in nature and technology. In: Muzzarelli R, Jeuiaux C, Gooddy GW, editors. Chitosan effect in nature and technology. New York: Plenum Press; 1986. p. 435–42.

[23] Li L, Hsieh YL. Chitosan bicomponentnanofibers and nanoporous fibers.Carbohydr Res 2006;341:374–81.